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Influence of 70 nm silica particles in mice with cisplatin or paraquat-induced toxicity

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In the pharmaceutical industry, nano-size materials are designed as drug carriers and diagnosis probes. Interactions between nano-size materials and chemicals need investigating. Here, we investigated whether nano-size materials affect chemical-induced toxicity using silica particles, which have been widely used in cosmetics and drug delivery and have diameters of 70 (SP70), 300 (SP300) and 1000 (SP1000) nm, a popular anti-tumor agent, cisplatin, and a widely used herbicide, paraquat. Mice were treated with either cisplatin (100 μmol/kg, intraperitoneally) or paraquat (50 mg/kg, intraperitoneally), with or without intravenous silica particle administration. All treatments were non-lethal and did not show severe toxicity, except for injection with both cisplatin and SP70, which were lethal. When mice received with paraquat and/or the silica particles, synergistic enhanced toxicity was observed in both paraquat- and SP70-treated mice. These synergic effects were not observed with either Si300 or 1000 treatment. Our findings suggest that further evaluation on the interaction between nano-size materials and chemicals is critical for the pharmaceutical application of nanotechnology.

1. Introduction

Nano-size materials are typically defined as engineered structures having at least one dimension of 100 nm or less. Changing from micro- to nano-size in materials expands surface area. In addition, nano-sized materials may have unique physicochemical properties due to their small size, chemical composition, surface structure, solubility, and shape. Recent development of nano-size particles from the micro- to nano-scale provides us with new tools, not only for industrial use such as electronics and catalysts, but also for pharmaceutical use such as cosmetics, diagnostic imaging and drug delivery (Caruthers et al. 2007; Vallet-Regi et al. 2007; Bartlett et al. 2007; Medarova et al. 2007). Although an expanded surface area of nano-size materials is advantageous, wide surface area can be accompanied by increasing interactions with biological tissues, cells, proteins, and nucleic acids, leading to toxic effects on humans (Nel et al. 2006; Oberdorster et al. 2005; Fischer and Chan, 2007). It is rare for a human to be exposed to only nano-size materials; we are often exposed to nano-size materials as well as other substances, such as xenobiotics and pharmaceutical agents. Nano-silica particles are intended for cosmetics and systemic and local delivery of drugs (Vallet-Regi et al. 2007). Previously, we found that intravenous administration of 70 nm, but not 300- and 1000 nm, silica particles caused liver injury (Nishimori et al. in press). Taken together, the synergistic effect of nano-size materials with other toxic substances should be evaluated, as there are few studies to date.

In this study, we investigated the synergistic effect of 70 nm silica particles with chemicals using cisplatin, a widely used anti-tumor agent (Ozols and Young 1991; Hartmann et al. 1999; Witjes 1997), and paraquat, one of the most widely used and highly toxic herbicides (Vandenbogaerde et al. 1984), providing evidence for synergistically enhanced toxicity.

2. Investigations and results

We previously found that intravenous administration of 70 nm size silica particles (SP70) caused liver failure, but 300- (SP300) and 1000- (SP1000) nm size particles did not (Nishimori et al. in press). Here, we investigated whether interaction between chemicals and silica particles occurred. To avoid direct interactions between chemicals and silica-particles before administration and absorption, we injected chemicals and silica-particles intraperitoneally and intravenously, respectively. Administration of cisplatin has been shown to cause adverse effects such as hepatic and renal failure (Lu and Cederbaum 2006; Ramesh et al. 2007). Indeed, serum levels of biochemical markers for hepatic and renal injury were elevated by cisplatin as shown in Fig. 1A and B, respectively. Co-treatment with cisplatin and SP300/1000 did not exhibit severe toxicity, whereas co-administration with cisplatin and SP70 showed lethal toxicity. Although SP70 did not show hepatic toxicity at 20 mg/kg, co-administration of SP70 with cisplatin caused the death of 5 of the 8 mice (Fig. 1). The surviving

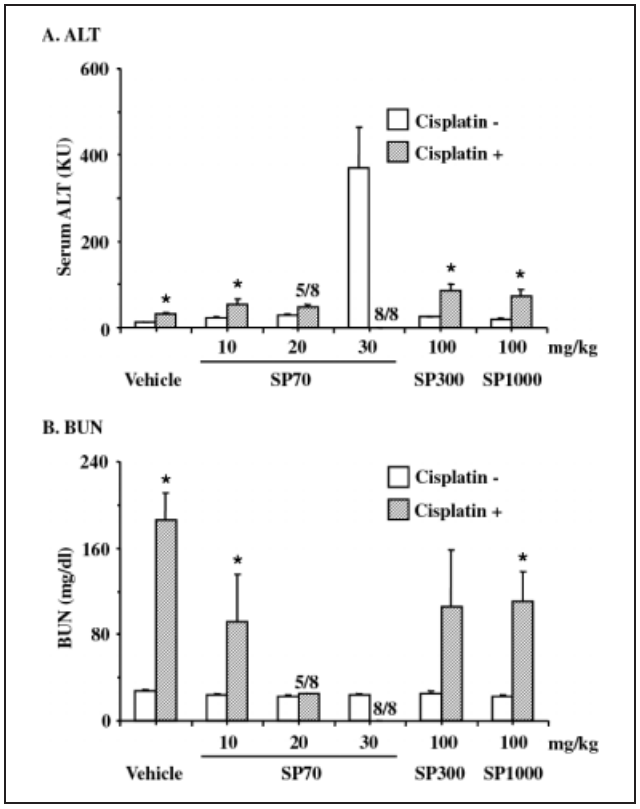

Fig. 1: Effect of SP70 on cisplatin-induced toxicity
Mice were injected with cisplatin at 0 (open column) or 100 μmol/kg (filled column) and each silica particle (SP70, 70 nm particles; SP300, 300 nm particles; SP1000, 1000 nm particles) at the indicated dose, intraperitoneally and intravenously, respectively. At 24 h post-injection, the serum was recovered. ALT (A) and BUN (B) levels were assayed as described in the Materials and methods. Five of 8 and 8 of 8 mice died in the 20 mg/kg SP70/cisplatin and 30 mg/kg SP70/cisplatin-injected group, respectively. Data are representative of three independent experiments. Data are mean ± SEM (n = 4–12). *Significant difference between vehicle and cisplatin-treated group ($p < 0.05$)

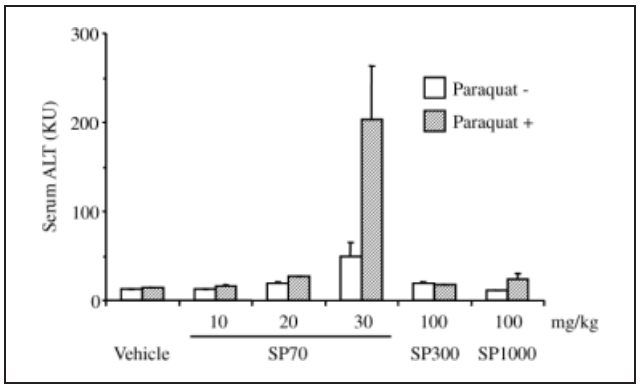

Fig. 2: Effect of SP70 on paraquat-induced toxicity
Mice were injected with paraquat at 0 (open column) or 50 mg/kg (filled column) and silica particles (SP70, SP300 or SP1000) at the indicated dose, intraperitoneally and intravenously, respectively. At 24 h post-injection, the serum was recovered. ALT levels were assayed as described in the Materials and methods. Data are representative of two independent experiments. Data are the mean ± SEM (n = 4)

mice did not show abnormal ALT and BUN levels. SP70 did not show a lethal effect on mice at 30 mg/kg, but all mice injected with SP70 (30 mg/kg) died. We next investigated the interaction between paraquat and silica particles. Co-administration of paraquat (50 mg/kg) and silica particles did not elevate serum ALT, and SP70 showed synergistic elevation of serum ALT levels from 48.9 to 203.4 KU (Fig. 2). Synergistic effects of paraquat on SP300 or 1000 were not observed.

3. Discussion

In this study, we investigated the combined effects of chemicals on nano-size particle-induced toxicity, and found cisplatin and paraquat had synergistic toxic effects with silica particles with a diameter of 70 nm.

One characteristic of nano-size materials is their large surface area, and one explanation for the differing synergistic effects of nano- and macro-size particles is this difference in surface area. Indeed surface areas of SP300 and 1000 are 0.229 and 0.0068 per gram of particles relative to that of SP70, respectively. Lethality and ALT levels in cisplatin and paraquat, respectively, were observed with the injection of SP70 at 20 mg/kg, but not SP300 at 100 mg/kg, at which point the surface area of SP300 is almost equal to that of SP70. Therefore, the influence surface area has on the additive toxicity may be negligible. We previously found that differing hepatotoxicity among SP70, 300 and 1000 might be due to the accumulation of SP70 in the Disse space between liver sinusoidal endothelial cells and hepatocytes (Nishimori et al. in press). Differences in silica particle dynamics may be responsible for the synergistic effects of SP70. The profile of absorbed proteins on silica particles differed with particle size, and the amount of absorbed proteins was dependent on surface area; i.e., smaller particles absorbed more proteins on the surface (Dutta et al. 2007). The most abundant serum protein is albumin, and chemicals absorbed into the systemic circulation are often absorbed onto albumin. The structurally altered albumin is rapidly cleared from the circulation by a scavenger receptor (Demoy et al. 1999; Jansen et al. 1991; Kamps et al. 1997). The albumin-chemical complexes may be absorbed onto SP70, resulting in the aggregation of chemicals onto SP70 particles through albumin. Lipid-coated aggregation of cisplatin increased the cytotoxicity to 1000-fold compared with free drugs (Burger et al. 2002). The aggregated particles containing high dose of chemicals might be taken up, leading to enhanced toxicity. We will perform further biochemical and comprehensive analyses, such as proteome and genome assays, to determine the mechanism of these synergistic effects.

This report indicates synergistic toxicity of a nano-size silica particle with chemical agents. Further evaluation of such interactions between nano-size materials and pharmaceutical agents for future pharmaceutical application of nanotechnology are necessary.

4. Experimental

4.1. Materials

Silica particles with a diameter of 70, 300, or 1000 nm were obtained from Micromod Partikeltechnologie GmnH (Rostock, Germany). The size distribution of the particles was analyzed by a Zetasizer (Sysmex Co., Kobe, Japan), and mean diameters were 55.7, 296, and 989 nm, respectively. The particles were spherical and nonporous, and stored at 25 mg/ml (70 nm) and 50 mg/ml (300 and 1000 nm) in aqueous suspension. The suspensions were thoroughly dispersed with sonication before use and diluted in water. An equal volume of solution was injected in each treatment. Paraquat and cisplatin were dissolved in saline and stored at −20 °C before use. All reagents used were of research grade.

4.2. Animals

The 8-week-old BALB/c male mice were purchased from Shimizu Laboratory Supplies Co., Ltd. (Kyoto, Japan). They were maintained in controlled

environment (temperature: $23 \pm 1.5\,^{\circ}C$; light: 12 h light/dark cycle) with free access to standard rodent chow and water. The mice were given 1 week to adapt before commencing. The experimental protocols conformed to the ethical guidelines of the Graduate School of Pharmaceutical Sciences, Osaka University.

4.3. Biochemical analysis

Serum alanine aminotransferase (ALT) and blood urea nitrogen (BUN) were measured using commercially available kits according to the manufacturer's protocols (WAKO Pure Chemical, Osaka, Japan).

4.4. Statistical analysis

Statistical analysis was performed by Student's t-test. $P < 0.05$ considered statistically significant.

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